THE PERFUSION OF SURVIVING ORGANS.

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THE method of examining the physiological action of an organ or tissue by perfusing it by blood after its removal from the body has already proved of great value in many instances, and under proper conditions can solve many physiological problems. The methods hitherto employed possess several great disadvantages, of which the most marked are the necessity of employing large volumes of blood, and the absence of any satisfactory provision of a method of aëration. It is not necessary to refer in detail to all the various forms of perfusion apparatus that have been devised by different workers since Ludwig and Schmidt[1] first described the apparatus they had constructed for the perfusion of isolated surviving mammalian muscle. I need only refer to that of v. Frey and Gruber[2] in which special arrangements were provided for quantitatively determining the changes of the blood gases in the perfusion, and to the elaborate apparatus of Jacobi[3], in which a double circulation was maintained—the one through the organ which was being studied, and the other through an isolated lung in order that the blood might be continuously oxygenated. Many workers have simply used two flasks, which alternately fed the artery, the blood being driven from them by air pressure. The great objection to most forms of apparatus hitherto employed is the necessity of obtaining a large supply of blood, the animal's own blood being, in the majority of cases, insufficient.

In attempting to overcome these various disadvantages I have tested many modifications of previously described apparatus, and as

[1] Ludwig and Schmidt, *Leipzig Ber.* xx. p. 12. 1868.

[2] V. Frey and Gruber, *Arch. f.* (*Anat. u.*) *Physiol.* p. 518. 1885.

[3] Jacobi, *Arch. f. exp. Path.* xxxvi. p. 330.

a result of these experiments I have finally adopted the following as best fulfilling the essential requirements. The general plan is given in the accompanying diagram (Fig. 1). It consists of a pump *D*,

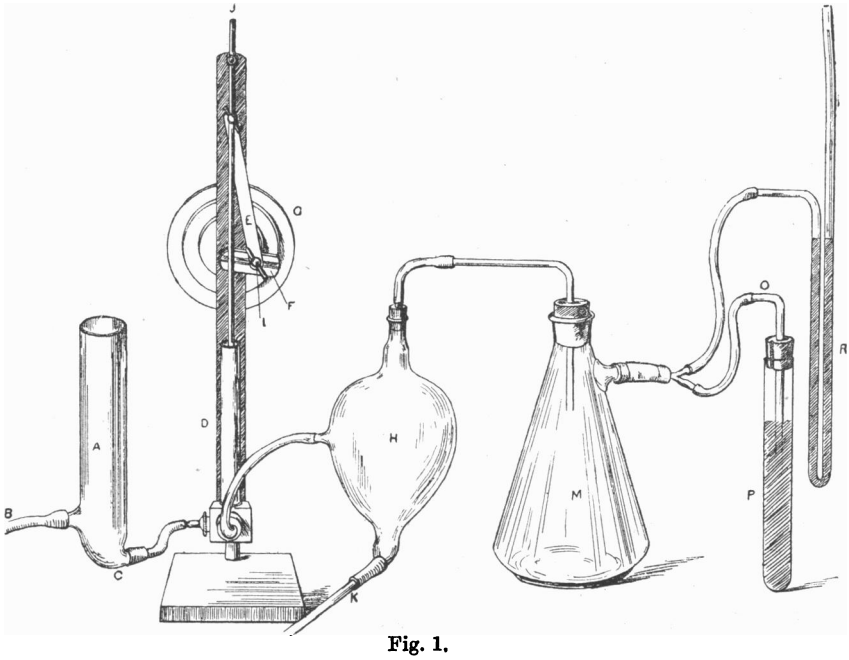

Fig. 1.

driving the blood into a glass receiver *H*. The tube *K*, at the bottom of this receiver is connected by rubber or glass tubing to a cannula tied in the artery of the organ to be perfused. The second cannula tied in the vein is connected by rubber tubing to a lateral inlet *B*, in the glass vessel *A*. The bottom of the vessel *A* is provided with a second tube *C*, which is connected to the inlet tube of the pump *D*, and the outlet tube of the pump is connected to a lateral tube of the receiver *H*. The pump consists of a piston and cylinder, the piston rod *J* being moved by a crank *F* and connecting bar *E*. The pump is provided with cylinders and pistons of different diameters so that it can be used for organs of varying sizes and for various rates of flow of blood. The amount delivered by the pump at each thrust can be further regulated, for the crank which is fixed on an axis driven by a coned-pulley *G* is slotted, so that the connecting bar *E* fixed at the one end of the piston-rod can, at its other end, be clamped in any position in the crank by the screw *I*. In this way the thrust of the crank can be quickly

adjusted to move the piston through any desired length and thus vary the volume of the fluid delivered per thrust. The valves of the inlet and outlet tubes of the pump must be both gas and fluid tight. After testing a very great number I have found the best for this purpose are made in the following way: A short length of thin-walled rubber tubing about 5 mm. in diameter is tied on the end of the delivery tube, and into the other end a small double-convex glass lens about 15 mm. in diameter is inserted; the thickness of the wall of the rubber tube is only 0·5 mm., so that it is well stretched out by the glass lens. This valve permits fluid and air to pass between the rubber and the lens but is absolutely air and fluid tight in the reverse direction. It possesses the great advantage that it is quickly made or renewed and can be readily cleaned. It is fitted into a short length of glass tubing which is wide enough to receive it, and this in turn is connected to the receiver *H*. A similar valve is fitted on the inlet tube of the pump, working, of course, in the same direction. The upper end of the receiver *H* is closed by a cork through which a glass tube passes; this is connected by rubber tubing to a large flask *M*. By a side-tubular and *Y*-piece the flask is connected to the mercury manometer *R*, and a mercury valve *P*.

The two receivers and the pump are fixed in a convenient water-bath maintained at body temperature.

The mode of working is as follows: The glass receivers and pump having been thoroughly cleaned by pumping physiological saline solution through them, the tubes *B* and *K* are clamped, the animal is anæsthetised and the artery and vein of the organ to be perfused are next isolated, and loose ligatures having been passed round them the animal is bled to death, its blood collected, whipped, filtered through glass-wool and then poured into the receiver *A*, whence it is at once pumped into the main receiver *H*. The mercury valve having been set at the required pressure the pumping is continued until air escapes through this valve so that the blood in the receiver *H* is under the initial pressure required for the perfusion. While the blood is thus being transferred to the receiver by an assistant, the cannulæ are quickly tied in the artery and vein. The artery cannula is then connected to the receiver by the tube *K*, care being taken that this tubing and the cannula are quite filled with blood and that no air is included. A short length of rubber tubing is fitted to the cannula in the vein and the clamp on *K* is then removed. The first blood that flows out through the vein is collected in a small beaker, whipped,

filtered and then returned to the vessel *A*. After a fair amount of blood has issued from the vein, that is, a quantity sufficient to wash out the blood originally retained within the organ, the venous cannula is connected directly to the lateral tube *B* on the receiver *A*. The pump is now kept running continuously, so that the blood as it collects in *A* is at once driven to the main receiver *H*. The thrust of the pump and its rate are then regulated so that enough air is pumped with the blood to sufficiently aërate it. The air in excess of that required to maintain the pressure at a constant height passes through the vessel *M*, and escapes through the mercury valve *P*. The object of the vessel *M* is to serve as a froth chamber, for it is found that some bloods froth considerably, and if froth is not collected in this way it escapes through the mercury valve, thus interfering with its efficient action.

In many cases it is advisable to vary the pressure of the blood-supply rhythmically in imitation of the pulsation of an artery. This is easily effected by replacing tubing *K* by a fairly distensible piece of rubber tubing passed under a clip. This clip consists of two wooden

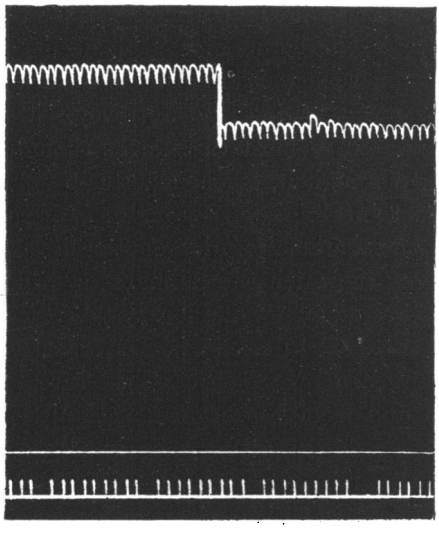

Fig. 2. Record of variations in lateral pressure of the blood perfusing an excised kidney. At the centre of the tracing the mean pressure was suddenly reduced. Time in seconds. The line above the time tracing is the zero of blood-pressure.

arms between which the tubing passes in a transverse direction. The upper arm is pulled down by a spring so as to compress the tubing and

is rhythmically lifted by a rotating cam, to allow the blood to flow from the receiver into the partially collapsed tubing on the other side of the clip. By varying the shape of the cam and the rate at which it is driven a fairly accurate reproduction of the variations of arterial blood-pressure can be obtained in the peripheral tube and artery. A record of these pressure changes taken by a mercury manometer is given in Fig. 2.

This apparatus has now been in constant use for the past four years, during which time small details have been gradually altered or added, as experience has taught their necessity. There are also many other points of technique the observance of which is of great importance. These are considered in the following sections:

The preparation of the blood. In my earlier experiments I commonly employed fresh defibrinated sheep's or ox blood in perfusing organs taken from cats or dogs, but it gradually became apparent that in very many cases this was quite inadmissible. Newell Martin in his experiments upon the isolated dog's heart employed calf's blood, but he mentions that in several instances this blood was toxic to the dog's heart. In some of our earlier experiments upon the dog's heart we tried ox blood, sheep's blood and horse's blood, but found that as soon as the isolated foreign blood was supplied to the heart the beat became irregular. The heart next went into fibrillary twitchings, from which it could not be recovered in most cases, even though the animal's own blood was perfused through it. When perfusing other organs I have often observed that the rate of flow through the organs is usually much less when blood from an animal of different species is used, as compared to other experiments in which the animal's own blood is used. It is much better therefore to only employ blood taken from animals of the same species as that experimented upon, and best of all to make use only of the animal's own blood. With care in economising the tubing leading to and from the organ it is quite possible to perfuse most of the organs of the cat, using the animal's own blood only. In the case of the dog, where the yield of blood is much greater, it is always possible for all organs. In some organs, heart, limbs, etc., it is permissible to perfuse with blood diluted with normal saline solution, tap water saline, or Ringer's solution. This has already been employed by Langendorff and his pupils for the heart, and gives excellent results. Locke has indeed gone a step farther and employed oxygenated Ringer's solution only, and shown that the rabbit's heart will beat for hours when perfused in this way. In most organs and

tissues, however, and notably in the lungs, dilution of the blood with saline leads to the production of considerable œdema.

The difficulties in the way of obtaining a sufficiently rapid rate of flow through the perfused organ. The slow rate of flow commonly observed in perfusing an organ is usually ascribed to a persistent tonus of the arterioles of the organ, and this undoubtedly partly explains the phenomenon. This tonus is partly due to the sudden pressure to which the arteries are subjected in commencing the perfusion, and is especially marked in the case of the limbs or the kidney[1]. In the latter organ it is usual to obtain a good flow as soon as the perfusion is started, but this falls off to about one-third of its initial rate in about twenty to forty seconds, and commonly never increases again. To avoid this difficulty it is best to start the perfusion with a low pressure and then to gradually increase it until it reaches a normal height[2]. Even by this procedure the difficulty is by no means avoided and at times leads to a complete failure of the experiment. It has been shown for the lungs, by de Jaeger and others, that the rate of flow is greater if the pressure is made to rise and fall in imitation of the natural pressure. I have found that this is also true for some other organs, *e.g.* kidney, limbs, spleen, but the difference in rate of flow under this condition as compared to the flow under constant pressure is never very marked, and it is doubtful whether the advantage gained, except perhaps in the case of the kidney, compensates for the extra complication introduced in the apparatus.

There are, however, other causes by which a hindrance to the flow may be produced. The first of these is embolism, which may be due either to air or to foreign particles. If many air-bubbles escape into the artery the flow at once falls off, but it is remarkable how, in quite a short time, these may be washed through the capillaries if more air-bubbles are prevented from entering. In setting up a connection between the reservoir and the artery it is wise to introduce at some part a trap in the shape of a glass *T*-piece through which the blood is made to travel, the limb which acts as the air trap being set vertically and closed with rubber tubing and a pinch-cock. This limb may then from time to time be opened, thus driving out any collected air contained in it. A second possibility is that small solid particles may block the arterioles. These usually arise from the interior of the pump and containing vessels. It is quite impossible to so completely

[1] See in this connection Bayliss' paper. This *Journal*, xxvi. p. 220. 1902.

[2] This is not important in the case of the liver.

clean the pump and receiver that saline solution pumped through issues absolutely free from particles at the outflow. In some experiments in conjunction with F. S. Locke, we found this so important that we now invariably filter the blood through two glass-wool filters, one placed in the vessel receiving the blood from the vein, and a second small filter consisting of a fairly wide glass tube plugged with glass-wool inserted on the course of the tubing leading to the artery. This addition to the detail of an experiment has proved of enormous advantage, and in our opinion should never be omitted.

There is one other factor which I believe plays a part in the production of the arterial tonus, which is that serum is by no means a completely inert fluid. I have shown that in the cat the process of clotting produces substances which act as irritants to the pulmonary nerve-fibres, and working upon the hypothesis that a similar cause may be at work exciting the muscular walls of the small blood vessels, I have carried out perfusions with blood that has been prevented from clotting in various ways. As a result of these experiments there is no question as to the truth of this hypothesis, and one of the most effective bloods is citrated blood. The blood is received into a strong sodium citrate solution used in quantities sufficient to prevent clotting. Such a blood may be perfused for several hours without clotting, and unquestionably flows through the vessels more freely than defibrinated blood. When experimenting upon dogs I have also tried leech-extract blood. This again works favourably, but has the great disadvantage that a considerable quantity of the extract is required and frequently the experiment is lost by the formation of a clot. In the majority of cases the increased flow does not compensate for the risk of injury that is run by the addition of a foreign substance. The same holds for the increase produced by the addition of chloral, cocaine etc., to the blood.

The production of œdema. Attention has frequently been drawn to this point by previous workers, who have found that it is less liable to occur if the supply of blood be pulsating instead of at constant pressure. This I can also confirm when working under the ordinary methods, but I think that its avoidance is best attained by paying attention to two points I have already insisted upon, viz. to use only the blood of the same animal, and to avoid keeping the organ a considerable time without a blood-supply. The latter point is, in my opinion, by far the most important. I have commonly found the organ as normal in appearance (microscopically as well as macroscopically)

at the end of a two to three hours' perfusion as the control organ taken immediately after death.

The oxygenation of the blood. As above mentioned this end is aimed at by mixing air with the blood in its passage through the pump. Unfortunately this has not always proved satisfactory. If too much air is admitted a great deal of frothing occurs in most cases, and this entails a serious loss of blood. The blood returning from the limbs is extremely venous in appearance, and most difficulty in this direction has been experienced in these experiments. In the case of the heart the same appearance is seen, but as the flow is very slow there is no difficulty in oxygenating it. The blood from the spleen or kidney is less venous, and is therefore easily dealt with. In the liver, some trouble is found as the volume of the blood to be oxygenated is so considerable. In lung perfusions the blood is easily kept fully arterial and the difficulty may therefore be overcome by using the lung for the purpose. This, however, means the trouble of a double apparatus, and also necessitates a considerable bulk of blood, more than is usually available. Although I have attempted to render this aëration more perfect by several modifications, the apparatus is to some extent deficient in this respect. Fortunately the aëration has proved sufficient in our experiments up to the present time, as will be seen from the results published in the papers of W. Bain and K. Grube which follow.

The arrangements made for keeping the organ at body temperature. In my earlier experiments the receivers, pump, and connecting tubing were all immersed in a large water-bath kept at body temperature, in which was also fitted a large air chamber to hold the organ to be perfused. In many ways this proved very inconvenient, and in later experiments I have only found it necessary to enclose the main receiver in a small water-bath, using a second for the organ if necessary. If the flow through the organ is considerable, as for instance in the case of the liver, all that is necessary is to protect the organ from cooling by warm, moist cloths or cotton-wool, but in cases where the flow is slow it is necessary to place the organ in a warm chamber, and I have found it most convenient to provide a special one for the purpose. In those cases in which the whole of the blood leaving the organ can be collected from a single vein, as in the spleen, it is best to immerse the organ in a bath of normal salt solution, maintained at body temperature. This, too, is the simplest procedure in the case of the heart. For the kidney and other organs, in which the venous

outflow from the surface is considerable, it is necessary to provide a separate jacketed warm chamber, into which the blood drains and from which it is sucked by the pump and returned to the main receiver. A very convenient and satisfactory arrangement for maintaining the temperature of the two baths is that devised by Locke, and demonstrated by him in his experiment on the isolated rabbit's heart, shown at the Turin Physiological Congress last year. This consists of a long copper rod passing through the side of the bath close to the bottom. On that part of the rod outside the bath is hung a Bunsen burner, by two holes in the metal chimney attached to it. By moving this nearer or farther from the side of the bath, the temperature may be readily regulated and maintained constant with very little watching.

General. There still remain for consideration one or two points in connection with the differences in the arrangements when different organs are to be perfused. When small organs, whose outflows can be completely collected through their veins, are to be experimented upon, it is unquestionably best to isolate them completely from the body and immerse them in a bath of warm saline solution. This is the case, for instance, with the spleen, the heart, or the intestines. In those cases in which a considerable venous outflow occurs at the surface of the organ, as when perfusing the hind limbs, kidney, or liver, the organ should be placed in a warm air chamber—this should be made of glass or porcelain and protected by a glass cover. But in many cases it is wiser to keep the organ *in situ* within the body. This is especially advisable in the case of the lungs or the liver, for they perfuse much better when their normal shape is retained by the support given by the thorax and diaphragm respectively. When perfusing the liver, I found that a considerable loss of blood often occurred if the blood were collected from the inferior vena cava immediately above the diaphragm, the cava being ligatured just below the liver. This was due to the free anastomosis of hepatic veins with diaphragmatic and other veins. I ultimately found it best to collect the blood from the right auricle. A cannula is tied in the auricular appendix, and loss of blood through the ventricle is prevented by tying a thick ligature tightly round the middle of the two ventricles. If now the feet and head of the animal be slightly elevated above the level of the heart and the outflow opens at a level lower than the auricle itself the blood drains away easily and very little loss occurs. In this plan it is best not to ligature the inferior cava below the liver, nor even to ligature the superior cava. A similar procedure can also be adopted with advantage

for the kidney, especially if both organs are being perfused simultaneously. In perfusing the liver through the portal vein it is essential to ligature the hepatic artery and advisable to remove the lungs and intestines. After the perfusion is started the animal may be immersed in a bath of warm saline, or the more simple and quite effective procedure of closing the abdomen and keeping the animal warm with cotton-wool may be adopted.

In perfusing the lung, I open the thorax along the left side, taking care to leave the right pleural sac uninjured. A cannula is then tied in the commencement of the pulmonary artery, and a second in the left auricular appendix. The two ventricles are then ligatured and a second ligature is tied tightly round the root of the left lung. The right lung can now be perfused, and if desired can be rhythmically inflated through the trachea with warmed air. In this way the perfused lung is well protected.

The condition of the blood after circulating through the apparatus only. Many control experiments have been carried out, chiefly by Bain[1]. It was found by him that the vulcanite and metal used in the construction of the pump, caused a distinct destruction of the red blood corpuscles. In order to avoid this the apparatus has been modified so that the blood only comes into contact with glass, rubber tubing, and a small surface of wood and leather in the interior of the pump. This pump differs from that illustrated in the diagram, and has a form suggested by Mr Horace Darwin of the Cambridge Scientific Instrument Company. By employing this pump the red corpuscles are much less injured, although they still suffer to a slight degree. The white corpuscles remain practically unaltered. The specific gravity is also unchanged. So far as I have been able to judge this slight injury to the blood exerts no harmful effect upon the organs perfused, except the kidney. In this organ the urine secreted nearly always contains hæmoglobin, a condition which in any case would be obtained when using defibrinated blood. In a few control experiments in which citrated blood was circulated this destruction of the red corpuscles was distinctly less.

The apparatus in its present form was made for me by the Cambridge Scientific Instrument Company.

[Towards the expenses incurred in these experiments a grant has been received from the Government Grant Committee of the Royal Society.]

[1] See his paper in the present number of this *Journal*.